NMR Data Acquisition

The process of data acquisition results in an FID signal residing in the computer of the NMR instrument. In order to properly set up the acquisition parameters, it is helpful to understand a little about how this is accomplished. The sequence of events involved in the acquisition of the raw data for a simple 1D ^{1}H spectrum on a 200 MHz instrument is as follows (Fig. 6):

1. Wait a period of time called the Relaxation Delay for spins to reach thermal equilibrium
2. Send a high-power short-duration radio frequency (200 MHz) pulse to the probe coil
3. Receive the resulting free induction decay (FID) signal from the probe coil
4. Amplify this weak RF (~200 MHz) signal
5. Convert the RF (MHz) signal to a "stereo" audio (kHz) signal
6. Sample the audio (analog) signal at regular intervals and convert it to a list of integers
7. Add the digital FID "list" to a sum FID in memory
8. Repeat steps 1-8 for as many "scans" or "transients" as desired

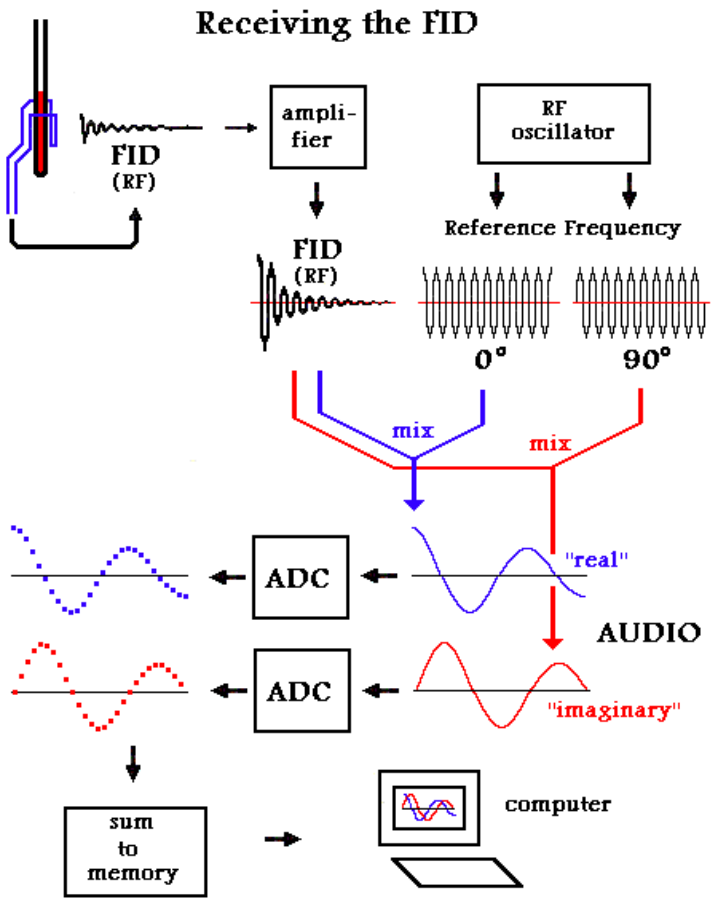


Figure 6. The precessing nuclei in the sample induce the radio frequency FID signal in the coil of the probe. This signal is amplified and mixed with two reference RF signals at the detector. The reference signal frequency determines the center of the spectral window. One reference signal is shifted in phase by 90° from the other. The result of the mixing step is an audio frequency which is the difference between the FID frequency and the reference frequency. This audio frequency can be either positive (downfield of the center of the spectral window) or negative (upfield). There are actually two audio signals, which are called the "real" and the "imaginary" parts of the FID, resulting from mixing with either a 0° or a 90° reference frequency. The analog-to-digital (ADC) converter samples these FIDs at regular intervals and sends a list of numbers to the Sum-To-Memory, which adds them to the running sum of FIDs. When all scans are finished, this sum is stored in the computer as the FID.

Each of these steps will be discussed in more detail, with the goal of understanding the basic parameters needed to set up an NMR experiment. The FID contains all of the frequencies of the sample protons, which represent a

range of chemical shift values. The range of radio frequencies in the FID is extremely narrow: 199.999 MHz to 200.001 MHz for a 10 ppm range of chemical shifts. We are only interested in this tiny slice of frequencies, so it is convenient to subtract out the fundamental frequency (200.000 MHz) and look only at the differences, ranging from -0.001 MHz to +0.001 MHz, or from - 1.000 kHz to +1.000 kHz. These much lower frequencies are called "audio" frequencies, since they are in the range of sound waves which can be detected by the human ear. In fact, the audio signal of an NMR can be connected to a pair of speakers so you can listen to the FID in stereo!

This subtraction of frequencies is accomplished by an electronic process called "mixing". It actually involves multiplication of the FID signal with a reference frequency signal (200.000 MHz in this example), with the resulting signal having frequency components representing both the sum and the difference of the FID frequency and the reference frequency.

199.999 - 200.001 MHz range X 200.000 MHz reference =

-1.000 kHz to +1.000 kHz
difference

399.999 - 400.001 MHz
sum

The sum is eliminated by an electronic filter, leaving only the desired audio signal. In case any of you are wondering about this bit of electronic magic, it can be explained by high school trigonometry as follows:

$$\sin(\alpha t)\cos(\beta t) = 1/2\ \{[\sin(\alpha t)\cos(\beta t) + \sin(\beta t)\cos(\alpha t)] + [\sin(\alpha t)\cos(-\beta t) + \sin(-\beta t)\cos(\alpha t)]\}$$
$$= 1/2\ \{\ [\sin((\alpha+\beta)t)] + [\sin((\alpha-\beta)t)]\ \}$$

This just says that the product of two waves of different frequency α and β is that same as the sum of two waves of frequency $\alpha+\beta$ (sum) and $\alpha-\beta$ (difference).

The audio signals must be converted into a list of numbers, which is the only language that a computer understands. This is done by sampling the voltage of each signal at regular intervals of time and converting each analog voltage level into an integer number. Thus an FID becomes a long list of numbers, which is stored in the computer memory. As the same FID is acquired over and over again, repeating the sequence: relaxation delay, pulse, acquire FID, each new list of numbers is added to the list stored in memory. This process is called "sum to memory". As more and more "scans" or "transients" are acquired, the signal-to-noise ratio of this sum improves. Now let's look in detail at each process, so we can understand the NMR acquisition parameters.

1. Relaxation Delay: **D1** (Varian) or **RD** (Bruker). Usually the acquisition (Pulse-FID) is repeated a number of times in order to sum the individual FIDs and increase the signal-to-noise ratio. In this case a delay must be inserted before each pulse-FID sequence to allow the populations to return to a Boltzmann distribution ("relaxation"). Without this delay the nuclei will become saturated (equal populations in the two energy levels) and there will be little or no signal in each FID. Ideally, you should wait about five times the characteristic relaxation time (T_1) before starting the next pulse, but in practice the relaxation delay is quite a bit less and you live with a certain reduction of signal. This is a compromise value because pulsing more often gives you more data per unit time. In this case, you rapidly reach a steady state where the nuclei

are not completely relaxed but are at least at the same degree of relaxation each time a new pulse arrives. Relaxation is going on during the FID acquisition period as well, and sometimes with long acquisition times there is no need for a specific relaxation delay. Another strategy for slow-relaxing nuclei (e.g., quaternary carbons in ^{13}C-NMR) is to reduce the pulse width so that the perturbation from equilibrium resulting from each pulse is reduced.

2. The Pulse (PW). The RF pulse is simply a high-power RF signal turned on for a very short period of time, on the order of microseconds (μs). The duration of the RF pulse in microseconds is called the Pulse Width (**PW,** Fig. **7**). The frequency of the pulse is set at the center of the spectral window (range of resonant frequencies expected), but because of its short duration it is capable of exciting all of the sample nuclei within the spectral window simultaneously. Using our example of a 200 MHz ^{1}H experiment, the pulse frequency would be 200.000 MHz but all nuclei resonating in the range 199.999 to 200.001 MHz would be equally excited by it. The pulse shape is rectangular, meaning that the RF power turns on more or less instantaneously to full power, and then PW microseconds later turns off. A "90 degree pulse" is the pulse width required to exactly tip the sample magnetization from the z-axis into the x-y plane (Fig. 7), where it rotates at the resonant frequency in the x-y plane, leading to a maximum

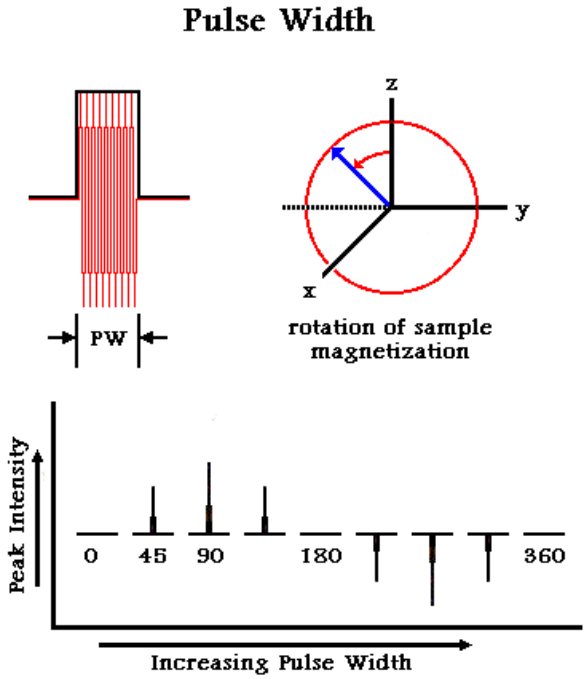


Figure 7. The pulse width (PW) is the duration of the excitation pulse in microseconds. The pulse is a high power RF signal which is turned on and then after PW microseconds turned off. The sample magnetization, which is on the Z axis at equilibrium, is rotated about the X axis by the excitation pulse. A "90 degree pulse" is the length of time it takes to rotate the magnetization to the -Y axis. Longer pulses will continue rotating to the -Z axis (180^{o} pulse) and to the +Y axis (270^{o} pulse).

intensity FID signal. The sample magnetization is just the sum of all the individual nuclear magnets. The length of the 90 degree pulse depends on the RF power, and to some extent on the probe and the sample. For some experiments, calibration of the 90 degree pulse width is essential for the experiment to work right. For simple one-pulse experiments an approximate value is sufficient. In more sophisticated experiments which use more than one pulse separated by various time delays, the pulse duration parameters are **P1**, **P2**, **P3**, etc. for the various pulses in the sequence. Pulses are always entered in microseconds (μs) and should not be made very long (more than a few hundred μs) because at full power the amplifiers can "burn up" if left on continuously for too long.

3. Receiving the FID from the sample. As the sample magnetization rotates in the x-y plane, the same probe coil which transmitted the high-power RF pulse to the sample experiences a very weak induced signal. This signal decays to nothing over a period of a second or two, and the full time course of this induced signal is called the Free Induction Decay, or FID. Each type

of nucleus in the molecule (e.g., the CH_3, CH_2 and OH protons in ethanol) has its own resonant frequency, so the FID consists of a superposition of a number of pure frequencies, corresponding to a number of peaks in the spectrum. All of the information of the NMR spectrum is contained in the FID, and a large part of the spectrometer is devoted to amplifying, recording and analyzing this signal.

4. The Receiver amplifies the radio frequency FID signal coming from the probe, converts it to an audio frequency signal by subtracting out the radio frequency at the center of the spectral window, amplifies it some more, and then converts it to a list of numbers. The total amplification given to the FID in the receiver is called the Receiver Gain (Varian: **GAIN** or Bruker: **RG**). The intensity of the FID signal induced in the probe coil depends on the sample concentration, so the amount of gain or amplification in the receiver must be adjusted for each new sample. The audio signal coming into the digitization stage (the analog-to-digital converter or ADC) should ideally be of the same magnitude for all samples, regardless of concentration. The ADC has a maximum range of integer values that it can give to the signal as it comes in, usually -32,767 to 32,768 (Fig. 8). If the signal is amplified too little before digitization, the

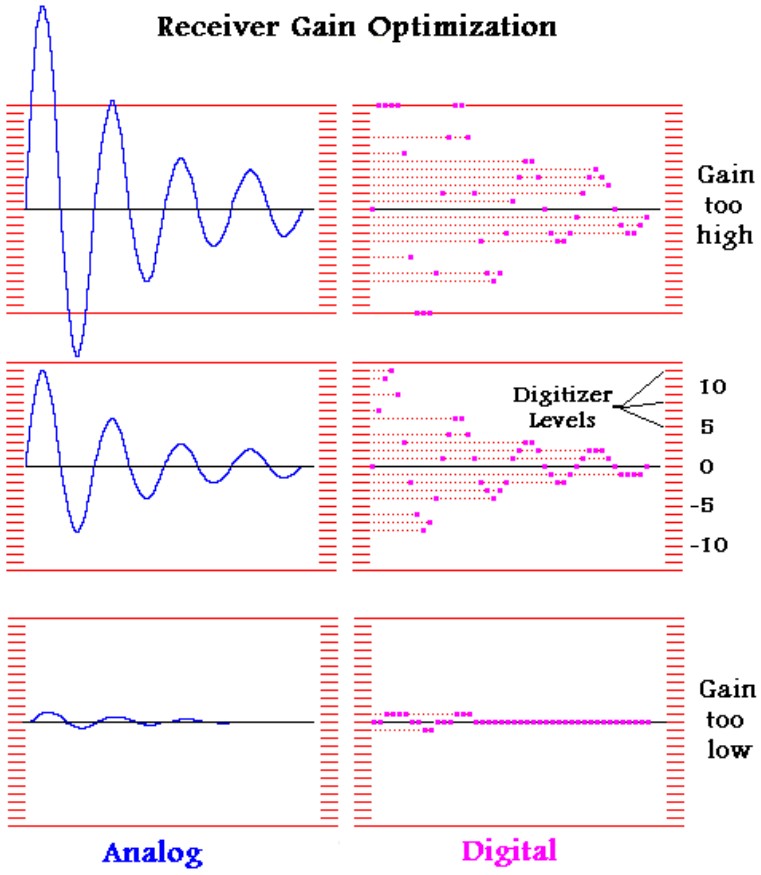


Figure 8. The ADC ("digitizer") can be viewed as a ladder of possible numerical values. In the figure integer values of -13 to +13 are shown, but in reality the range is usually more like -32767 to +32768. If the gain is set too high, the first portion of the FID has an analog voltage higher than the maximum of the digitizer, so these values are "clipped" at the maximum value. This will lead to large baseline rolls in the spectrum. The optimal gain just fills the digitizer with signal without exceeding the limits. If the gain is set too low, the shape of the signal is lost and a "blocky" representation results. This reduces signal-to-noise ratio and dynamic range.

numbers will get "grainy": they might range from -7 to +8 with only 16 possible values. In this case it would be very difficult to find a small peak in the spectrum in the presence of big ones (in fancy lingo, this is called a "dynamic range" limitation). If, on the other hand, if the signal is amplified too much, it might exceed the digitizer limits and get truncated or "cut off". For example, a signal which would give a value of 52,314 would be read as 32,768 because the digitizer can't respond to any larger value. This cutting off or "clipping" has very drastic effects on the spectrum: the baseline gets huge

oscillations ("wiggles") which can't be corrected in any way. So it's clear that the receiver gain has to be set correctly for each sample to get the best results. More concentrated samples (or samples with large solvent peaks) will require smaller receiver gain values, while dilute samples are best run with large gain. Both Varian and Bruker allow for an automatic receiver gain adjustment. On the Varian, simply set **GAIN** to 'N' (not used) and start the acquisition; a number of trial FIDs will be recorded to determine the best gain value and then the acquisition will begin. On the Bruker, the command **RGA** (receiver gain adjust) will do the same thing but will not automatically start acquisition.

5. The Detector converts the RF FID signal into an audio frequency FID signal by mixing it with a reference RF signal which has a single pure frequency at the center of the spectral window. The resulting audio signal has frequencies which represent the difference between the actual resonant frequencies of the sample nuclei and the reference frequency. This means that the audio frequency at the center of the spectral window is zero (reference freq. minus reference freq.); the downfield half of the spectrum represents positive audio frequencies and the upfield half represents negative audio frequencies. This corresponds to rotating the coordinate system used to represent the sample magnetization about the z (vertical) axis at the reference frequency. In this *rotating frame of reference*, a nucleus which resonates at the reference frequency would have its magnetization stand still in the x-y plane after the pulse. Nuclei which resonate in the downfield half of the spectral window have their magnetization rotate counter-clockwise in the x-y plane after the pulse, and those which resonate in the upfield half give rise to magnetization which rotates clockwise in the rotating frame of reference (Fig. 9). Placing the zero of our audio

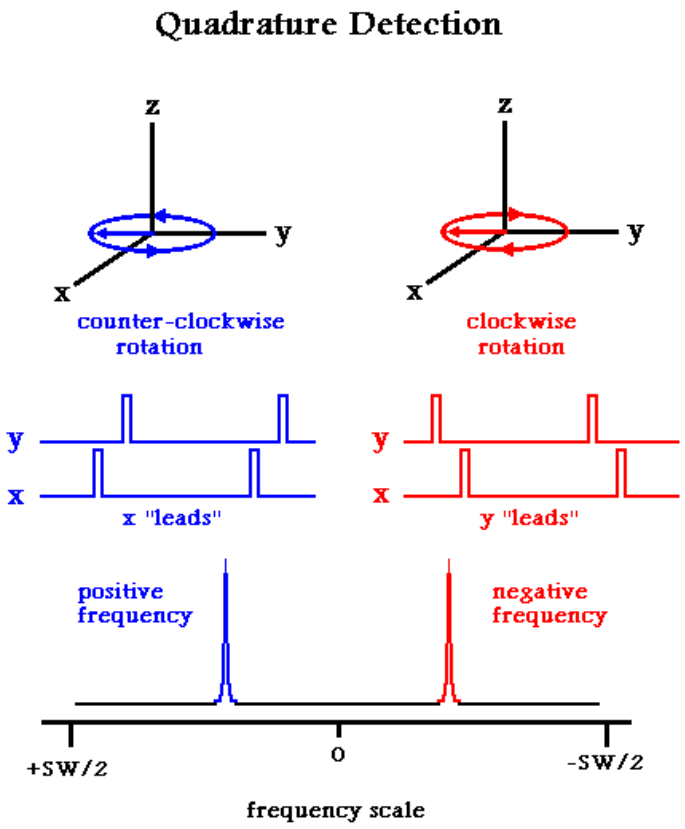
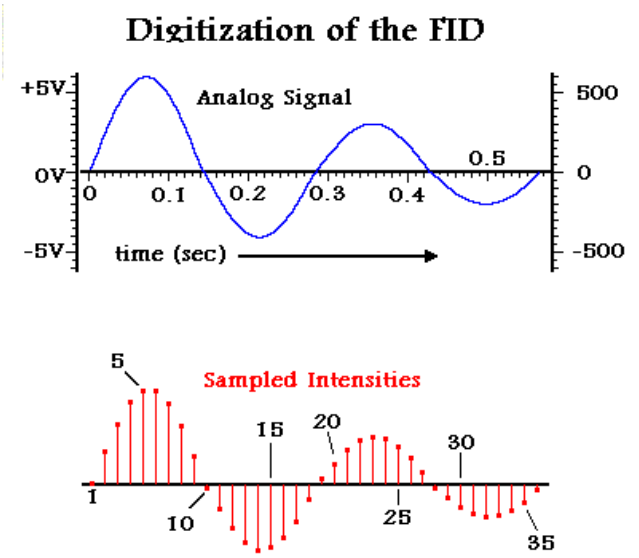


Figure 9. Relative to the reference frequency (center of the spectral window) the nuclei can rotate either clockwise or counter-clockwise in the rotating frame of reference. Imagine that we have a detector mounted on the x and y axes, and each time the magnet of the sample nuclei passes a detector it gives a "blip" from that detector. Clockwise rotation would give a pattern of blips where the y signal leads the x signal, while counter-clockwise rotation leads to a situation where the y signals lags behind the x signal. This is sufficient information to tell the direction of rotation, which is equivalent to the sign of the signal frequency. Thus the range of detectable frequencies is from -SW/2 to +SW/2.

frequency scale in the center of the spectral window requires that we have a way to tell the difference between positive frequencies and negative frequencies. This is accomplished by using a technique called **quadrature detection**. The radio frequency FID signal is mixed with two different reference RF signals, one of which is shifted by 90^{o} (one-fourth of a cycle) in phase with respect to the other (Fig. 6). The difference frequency is selected in both cases, resulting in two audio signals which are 90^{o} out of phase with each other. These signals are traditionally called the “real” and the “imaginary” FIDs, but there is nothing more or less real about either one. The best way to think about these “stereo” signals is to imagine that there are two coils in the spectrometer: one placed on the x axis of the rotating frame and one placed on the y axis of the rotating frame. If the magnetization resulting from a type of nucleus in the sample is rotating clockwise in the rotating frame, it will generate a signal in the y-axis detector just before it hits the x-axis detector, so that the “imaginary” (y-axis) signal will lag behind the “real” (x-axis) signal by 90^{o} (Figure 9). We can thus determine that this frequency is a negative audio frequency, and place the peak in the spectrum in the upfield half of the spectral window. Imagine a carrousel with one person riding on it near the edge. If you have two observers, one on the north side and one on the east side, and each observer calls out the direction as the rider goes by, you can tell which way the carrousel is rotating because you would hear “North East ... ... North East ... ...” for the clockwise direction and “East North ... ... East North ... ...” for the counter-clockwise direction. With only one observer you would hear, for example, “North ... ... North ... ...” and you would not be able to tell which direction the carrousel is rotating. The direction of rotation of the carrousel is analogous to the sign of the frequency of an NMR peak in your spectrum. If we only had one “detector”, which is the equivalent of having only one FID channel, we could not distinguish between positive and negative audio frequencies.

6. The Analog-to-Digital Converter (ADC). The computer cannot understand anything but numbers. The audio frequency FID is a continuous, smooth function of voltage (electrical intensity) *vs.* time. The analog-to-digital converter (ADC or digitizer) samples the FID voltage at regular intervals of time and assigns an integer value (positive or negative) to the intensity at each sample time (Fig. 10). These numbers go into a continuous list of numbers that constitute the digital FID. The spectrometer does not actually just acquire a single value for each time point - it's more like a stereo receiver. There are two channels in the receiver, one which effectively records signals along the "x" axis of the rotating frame and one which records signals along the "y" axis (Fig. 6). These are usually referred to as the "real" and "imaginary" parts of the signal, but this is just a mathematical convenience and doesn't imply that one channel is more real than the other. So the list of numbers is really twice as long because both FIDs are sampled by the ADC, and the numbers are loaded into the list in pairs: real(1), imag.(1), real(2), imag.(2), ..., etc. Bruker and Varian have different ways of sampling, which leads to some differences in processing and interpretation of data. Varian samples the two FIDs simultaneously at each time value, and Bruker alternates between real and imaginary samples in time; for example:



	time	value		time	value		time	value
1.	.0	0	13.	.192	-373	25.	.384	248
2.	.016	205	14.	.208	-416	26.	.400	179
3.	.032	386	15.	.224	-410	27.	.416	88
4.	.048	520	16.	.240	-354	28.	.432	-9
5.	.064	386	17.	.256	-126	29.	.448	-79
6.	.080	205	18.	.272	-256	30.	.464	-139
7.	.096	520	19.	.288	18	31.	.480	-183
8.	.112	386	20.	.304	113	32.	.496	-204
9.	.128	205	21.	.320	199	33.	.512	-201
10.	.144	-18	22.	.336	261	34.	.528	-174
11.	.160	-161	23.	.352	291	35.	.544	-125
12.	.176	-284	24.	.368	287	36.	.560	-62

Figure 10. Digital sampling of the FID leads to a list of positive and negative integers which describes the signal. Sampling occurs very rapidly and at a predetermined interval called the dwell time. In this example the dwell time was chosen to be 0.016 seconds or 16 ms. Because the values are assigned to integers, the analog intensities are rounded to the nearest integer at each sample.

	Varian (simultaneous)		Bruker (alternate)	
Time (μs)	Real	Imaginary	Real	Imaginary
0	1. 23435	2. -2344	1. 13465	
80				2. 9354
160	3. 6509	4. 3496	3. -3546	
240				4. 31593
320	5. 5673	6. -234	5. 23486	

Note that in 2D NMR the sampling in the second dimension can also be done either way, except that this choice is up to the user and is not "hard wired". The alternate ("Bruker-like") sampling method is called "TPPI" (for Time Proportional Phase Incrementation) and the simultaneous ("Varian-like") method is called "States" or "States-Haberkorn" (after the originators of the technique). The consequences for processing and interpretation of the data are the same in the second dimension as they are in 1D NMR.

This leads to confusion over two parameters: the number of points collected (do you mean the total number of data points, or the number of real / imaginary pairs?) and the time spacing between data points. Both Bruker and Varian list the number of data points (Varian: **NP**, Bruker: **TD**) as the total number of points, counting both real and imaginary. Some independent NMR software packages (e.g., Felix) count points as "complex pairs": one "point" corresponds to one pair of numbers (real and imaginary). The time spacing between successive data points sampled in the FID is called the dwell time (Bruker: **DW**). In the above example, the dwell time is clearly equal to 80 μs between samples for the Bruker data, but in the Varian case we need to think of the dwell time as the average time per sample, which is still 80 μs because two samples are collected in a period of 160 μs. Varian does not have a parameter corresponding to dwell time, leaving the sampling process hidden from the user. The two types of data (alternate and simultaneous) must be processed by a different Fourier transform method, but this is transparent as long as you process the data on the instrument which acquired it. If you transfer the data to a computer and use "third party" software (e.g., Felix) to process it, you need to choose the correct Fourier transform method for the type of FID data being processed.

How rapidly do we need to sample the data? Clearly this is limited by how fast the hardware can convert analog to digital, but in most cases this limitation is not serious. It turns out that the rate of sampling is determined by the highest frequency signal you need to describe by the digitized data. In other words, what peak in your spectrum is farthest from the center frequency (the reference frequency)? The highest frequency signal needs to be sampled at least twice during each cycle of its sine wave, meaning that the number of samples has to be twice the number of cycles in the highest frequency signal allowed.

$$\text{Number of Samples in 1 sec.} = 1 / DW = 2 * \text{Number of Cycles in 1 sec.}$$

$$1 / DW = 2 * F_{max}$$

Once we have chosen a particular dwell time DW, the maximum frequency we can accurately determine (since the computer doesn't know anything about the signal between the samples) is 1 / (2*DW). What happens if the frequency of a signal exceeds 1 / (2*DW)? The signal will not simply disappear; instead it is misinterpreted as a signal of lower frequency (Fig. 11). This process is called "aliasing" or "folding" because the peak appears at the wrong position in the NMR spectrum. Anyone who has watched Western movies or television shows has seen the phenomenon of aliasing. A film (or videotape) of a moving stagecoach will often show the wheels slowing, coming to a stop, or reversing direction even though the stagecoach is still obviously moving forward at full speed. The film is sampling the position of the spokes at a rate

of 30 frames (samples) per second. If the wheels move fast enough, the motion of the spokes exceeds the sampling rate and we interpret the motion as being a lower frequency than it really is. If this occurs in an NMR spectrum, we need to increase the sampling rate (decrease the dwell time DW) until we have two or more samples per cycle of the aliased frequency. Usually the aliased peak can be identified because it is lower in intensity and cannot be correctly phased.

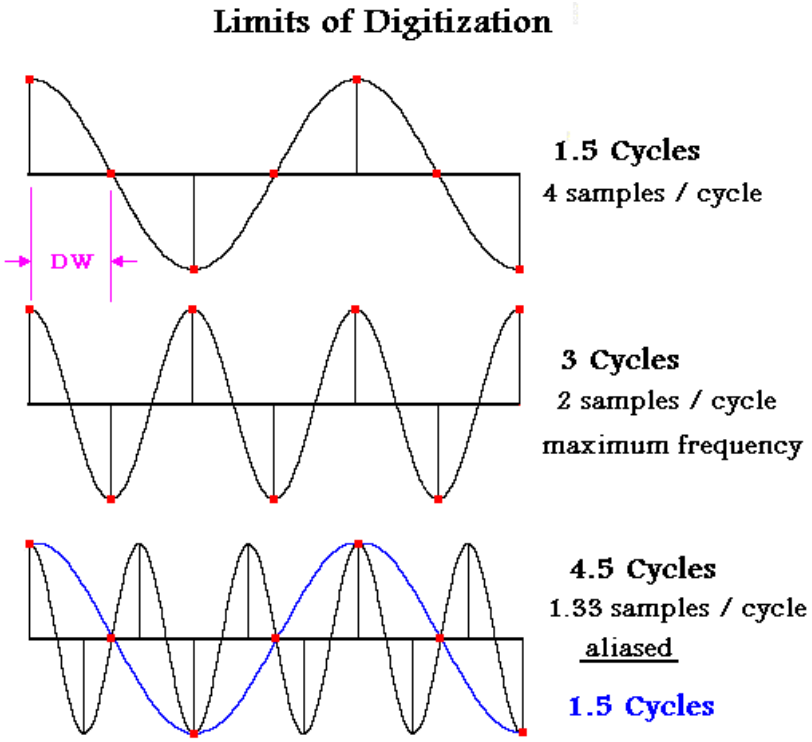


Figure 11. For a given interval between sampling ("dwell") frequencies can be accurately determined up to a frequency for which there are two samples per cycle. Lower frequencies have more samples per cycle, but higher frequencies have fewer than two samples per cycle. For example, the 4.5 cycle signal shown gives the same digitized data as the 1.5 cycle signal, and is thus interpreted incorrectly ("aliased") as a 1.5 cycle signal.

The limits of frequency imposed by a fixed sampling rate lead directly to the concept of the “Spectral Window” (Fig. 12). The center of the window is the zero point of audio frequency, which is determined by the reference frequency. The width of the spectral window is called the

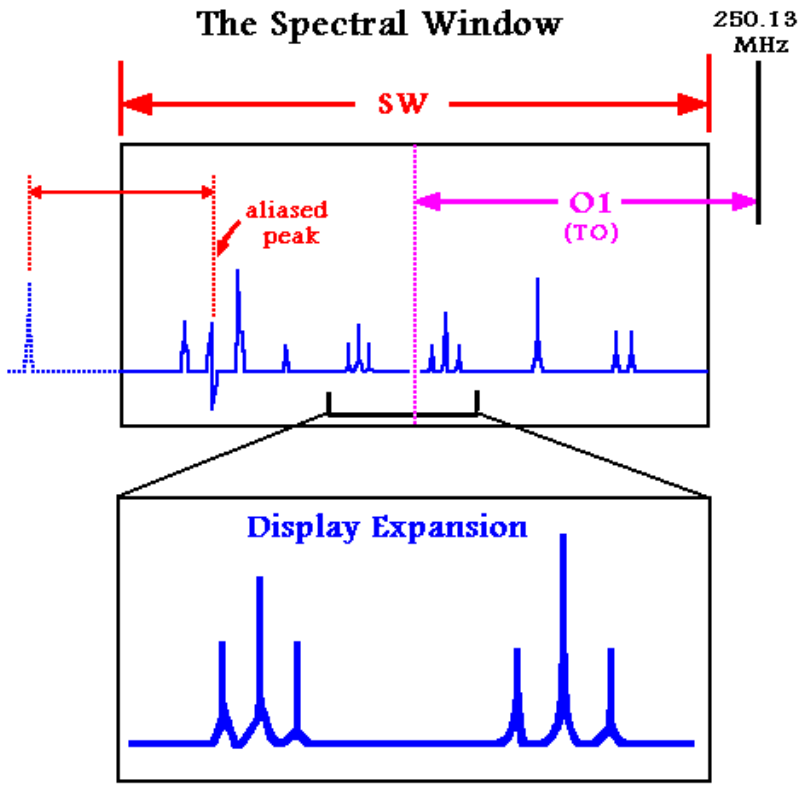


Figure 12. The reference frequency and the sampling interval or dwell (**DW**) determine the acquisition spectral window. Its width is the spectral width (**SW**) in Hertz and it is centered on reference frequency, which is adjusted by adding a variable offset (Bruker: **O1**; Varian: **TO**) to the fixed resonance frequency of the nucleus being detected (in this example, 250.13 Mhz for ^{1}H). A peak outside the spectral window will fold or "alias" into the spectral window, with anomolous phase. Any region of the spectral window can be expanded and displayed to fill the screen, but the spectral window is unchanged.

spectral width (SW), which is determined by the sampling rate. The spectral window can be moved to the left or right by adjusting the offset (Bruker: **O1**; Varian: **TO**) which changes the exact value of the reference frequency. The offset frequency is added to the fundamental resonance frequency for the nucleus of interest to obtain the reference frequency. For example, a 250 MHz instrument set up for proton acquisition might have a fundamental ^{1}H frequency of 250.13 MHz. Adding an offset (**O1**) of 10,000 Hz (0.01 MHz) would yield a reference frequency of 250.14 MHz. To move the spectral window downfield by 1 ppm (250 Hz), one would simply add 250 Hz to the offset value (**O1**), changing the value of this parameter from 10,000 to 10,250. Why would you need to move the spectral window upfield or downfield? The lock system changes the magnetic field strength of the spectrometer slightly to center the ^{2}H frequency of the solvent at the null point of the lock feedback circuit. Changing the field changes all of the resonant frequencies of the spectrum by the same amount, effectively moving the whole spectrum upfield or downfield by as much as 5 ppm when you change from one deuterated solvent (e.g., $CDCl_3$) to another (e.g., d_6-acetone). If this is not corrected by changing the offset by an equal and opposite amount, the spectrum will move out of the spectral window and some peaks will be aliased. For routine work, this hassle has been removed in two ways. On the Bruker, separate standard parameter files are provided for each lock solvent (e.g., PROTON.CHL, PROTON.D2O, PROTON.ACT, CARBON.CHL, etc.). These parameter files are identical except that the offset (**O1**) has been corrected for each solvent so that 5.0 ppm is in the center of the spectral window. On the Varian, the correction is made automatically by entering the lock solvent as the parameter "**SOLVNT**". This changes the fundamental resonance frequency so that the offset (**TO**) need be changed only for unusual samples with chemical shifts outside the standard (11 ppm to -1 ppm for ^{1}H) spectral window. This can be frustrating if you use the standard Varian parameters ($CDCl_3$) and neglect to change the **SOLVNT** parameter for solvents other than $CDCl_3$.

Thanks to the miracle of quadrature detection, the actual range of audio frequencies detected runs from +SW/2 to -SW/2, with zero in the center. Thus the maximum detectable frequency, negative or positive, is SW/2. The same arguments apply as to the relationship between the maximum frequency detectable and the dwell time, except that we are now dealing with complex pairs of numbers instead of single sample values:

Number of Complex Pairs in 1 sec. = 2 * Maximum Frequency Detectable

$$1 / (2*DW) = 2 * F_{max} = 2 * (SW / 2)$$

$$1 / (2 * DW) = SW$$

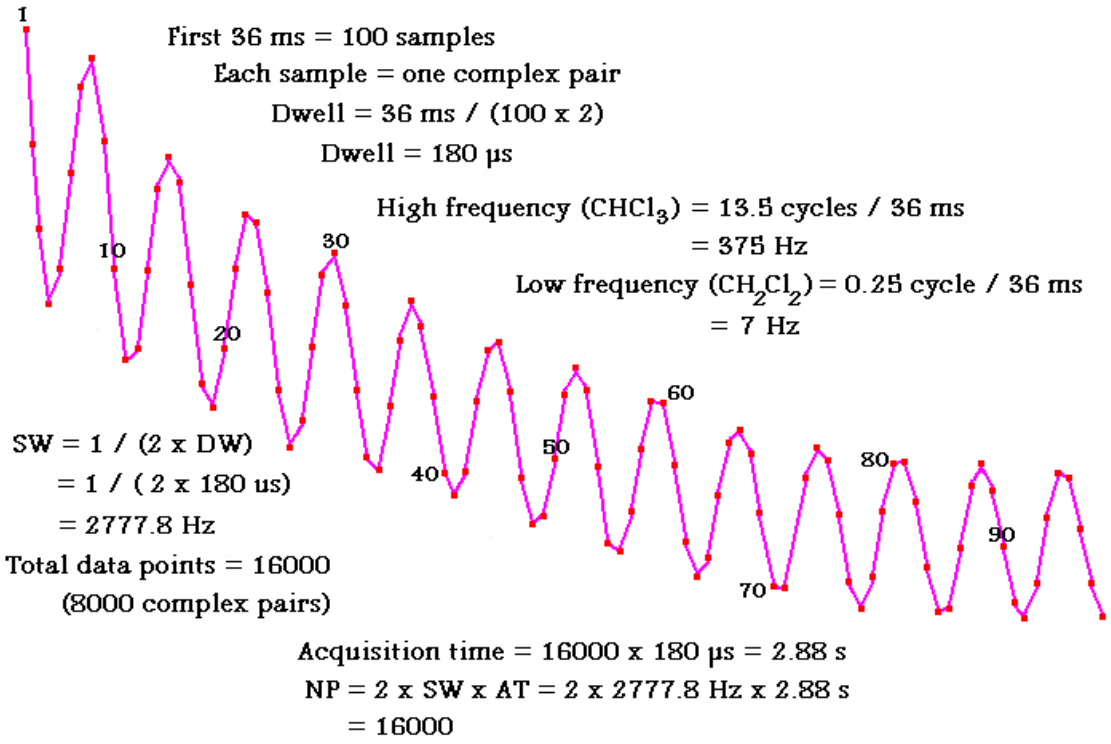
The last equation tells us what value of the dwell time we have to use to establish a particular spectral width. In practice, the user enters a value for SW and the computer calculates DW and sets up the ADC to digitize at that rate. The spectral window must not be confused with the "display window" which is simply an expansion of the acquired spectrum. The display window can be changed at will but the spectral window is fixed once the acquisition is started.

Any peak outside the spectral window will be aliased ("folded") into the spectral window at a position the same distance from the edge of the window. Aliased peaks are usually reduced in intensity (by electronic filters) and impossible to correctly phase; increasing the spectral width will eliminate them and reveal the peak in its correct position. The manner of aliasing depends on the type of acquisition. On the Bruker (alternating acquisition of real and imaginary data samples) aliased peaks appear reflected an equal distance from the same edge of the spectral window, as shown in Fig. 12. On the Varian (simultaneous acquisition of a real, imaginary pair of samples) aliased peaks appear an equal distance from the opposite edge of the spectral window.

> The same phenomenon applies to aliasing in the second dimension of a 2D spectrum: alternating (TPPI) acquisition in the second dimension will lead to aliasing on the same side of the spectral window; simultaneous (States) acquisition will lead to aliasing from the opposite edge of the spectral window.

An example of the real part of an actual FID of a sample of chloroform and dichloromethane (recorded on a Varian Gemini-200) is shown in Fig. 12. The sampled points have been connected by straight lines for clarity, but the data only records the individual points. The dwell time is 180 μs (only the first 100 real points are shown), leading to a spectral width (SW) of 1 / (2*180 μs) or 2777.8 Hz. The high frequency signal (from $CHCl_3$) has a frequency of 375 Hz, and the low frequency signal (from CH_2Cl_2) has a frequency of -7 Hz. The sign of the frequency can be determined only by examining the relative phases of the real and imaginary parts of the FID (quadrature detection). When you set the spectral reference using a standard such as TMS, you establish a third frequency scale (in addition to the absolute radio frequency scale and the audio frequency scale relative to the reference frequency) which is the chemical shift scale in ppm. Because the data was acquired on a 200 MHz spectrometer, an audio frequency of 200 Hz is 1 ppm away from the center of the spectral window. In this case the center of the spectral window is 5.37 ppm, so that the $CHCl_3$ chemical shift is 5.37 + (375 / 200) = 7.24 ppm and the CH_2Cl_2 chemical shift is 5.37 - (7 / 200) = 5.34 ppm.

Figure 12 **FID: $CHCl_3$ and CH_2Cl_2**



The 100 data points shown are part of a total FID of 8000 complex pairs (NP = 16000). Since a single data point takes 180 µs (the dwell time) to acquire on average, 16000 points require 16000 x 180 µs = 2,880,000 µs or 2.88 seconds to acquire. This is called the acquisition time (Bruker: **AQ**; Varian: **AT**), and it represents the time required to record the entire FID once. This is not the time required for the entire spectrum to be acquired, since it does not include the relaxation delay and the pulse width, and it doesn't take into account the number of times the whole sequence is repeated (i.e., the number of scans or transients). In general,

Acquisition Time = Number of points (real and imaginary) x Time required per data point

$$AT = NP * DW$$

But the dwell time (DW) is determined by the spectral width: DW = 1 / (2 * SW). Substitution of 1 / (2 * SW) for DW gives:

$$AT = NP * DW = NP * (1 / (2 * SW))$$

Rearranging:

NP = 2 * SW * AT (Varian)

TD = 2 * SW * AQ (Bruker)

Number of Data Points = 2 x Spectral Width x Acquisition Time

This is the fundamental equation of NMR data acquisition (the mnemonic "swat" is useful). It tells us that the three parameters NP, SW and AT (or TD, SW and AQ in Brukerese) are wedded by this equation such that changing any one of the three will require changing another to maintain the equality. For example, if we double the spectral width, either the number of points will double or the acquisition time will be cut in half. This is because the larger spectral width requires a faster sampling rate (half the dwell time) to assure that all of the frequencies in the spectral window are sampled at least twice in each cycle. With twice the sampling rate, you will either complete sampling the fixed number of points in half the time, or you will keep the acquisition time constant and sample twice as many points. Bruker keeps the number of points constant and changes the acquisition time; Varian leaves the acquisition time unchanged and calculates a new value for the number of points. This can be frustrating because parameters you thought you had not changed are changing before your eyes!

The spectrum resulting from Fourier transformation of this FID is diagrammed in Fig. 13. The three frequency scales shown illustrate the progression in recording the FID from radio frequency (actual frequency observed) to audio frequency (after subtracting out the reference RF signal) to a referenced chemical shift scale (after setting the spectral reference of TMS).

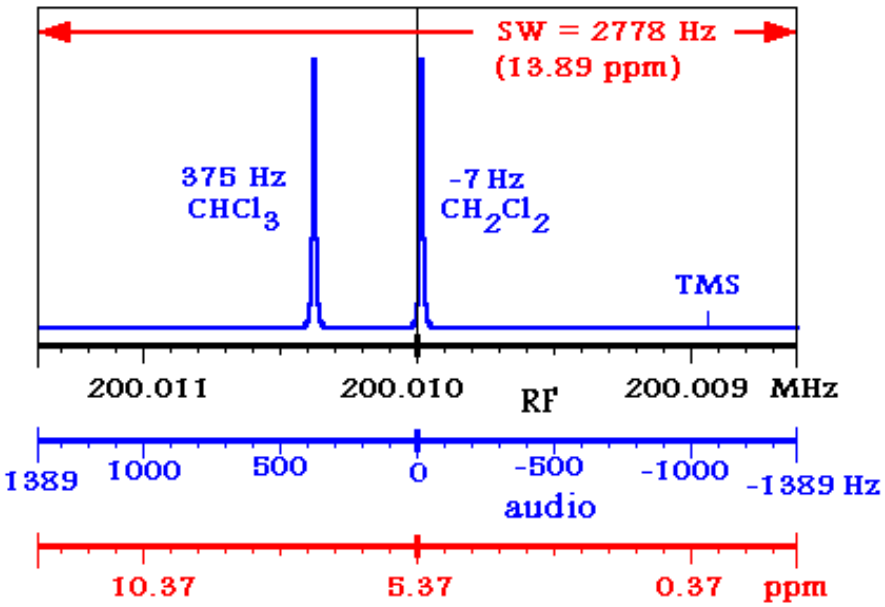


Figure 13. Spectrum obtained after tranformation of the FID shown in Fig. 12. The fundamental ^1H frequency is 200.000 MHz. The reference frequency is 200.010 MHz, obtained by adding an offset (**TO**) of 10,000 Hz to the fundamental frequency. After subtracting out the reference RF frequency, the audio frequencies range from -SW/2 to +SW/2 with zero in the center. The chemical shift scale in ppm is constructed from the spectral reference (TMS at 0.00 ppm) and the relation 1 ppm = 200 Hz.

7. The Sum to Memory. The sequence: [Relaxation Delay - Pulse - Acquisition of FID] is repeated a number of times with the acquired and digitized FID added each time to a "sum" FID stored in memory (Figure 14). Each individual FID contains the same signal (sum of decaying sine waves for all the sample nuclei) but the noise is different in each FID because it is random. The signal intensity increases directly with the number of repeats ("scans" or "transients"), but the

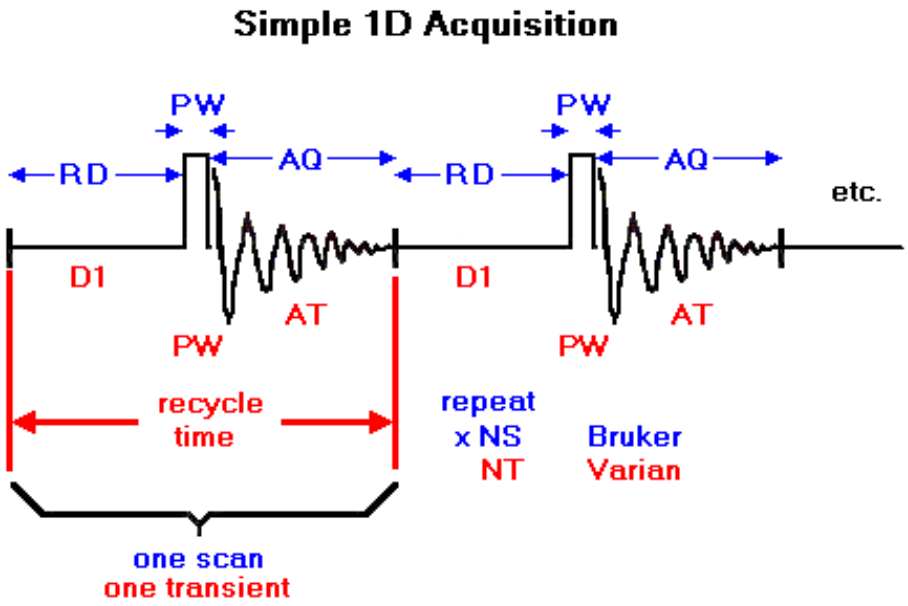


Figure 14. Simple 1-D Acquisition. The sequence RD-PW-AQ (Varian: D1-PW-AT) is repeated NS (Varian: NT) times, summing the FID to memory each time, for a total experiment time of NS*(RD+PW+AQ) or NT*(D1+PW+AT). One scan (or transient) is the single RD-PW-AQ (D1-PW-AT) sequence. The length of one scan is sometimes called the "recycle time".

noise increases with the square root of the number of repeats. This is like two people walking from the same starting point: one is sober and walks continuously in a straight line, and the other is drunk and changes direction regularly in a random fashion. The distance from the start is directly proportional to the time for the sober one, but the drunk walk is less efficient, gradually drifting farther and farther from the start. The signal-to-noise ratio ("S/N") is thus proportional to the number of scans divided by the square root of the number of scans:

$$S/N = \text{Signal} / \text{Noise} \quad \alpha \quad NS / (NS)^{1/2} = (NS)^{1/2}$$

This means that if you want to improve the S/N by a factor of two, you will need to acquire four times as many scans. Since the total experiment time is proportional to the number of scans:

$$\begin{aligned} \text{Time required} &= NS\ (RD + PW + AQ) \quad \text{(Bruker)} \\ &= NT\ (D1 + PW + AT) \quad \text{(Varian)} \end{aligned}$$

you will need four times as much time on the spectrometer to get a factor of two improvement in S/N.

Because in each repeated acquisition the observed data is simply added into the accumulated sum in memory, the size of the data file is not changed by increasing the number of scans. The individual FIDs are lost as their data values are added to memory. Consider a simplified example in which four FIDs are summed in memory. Although these calculations are always done in the computer with binary numbers, we will use decimal numbers in this example. Assume that the digitizer has available only one decimal digit (typically there are 16 binary digits) and that the memory allotment for each data point is two decimal digits (typically there are 16, 24 or 32 binary digits). Thus the FID data coming out of the digitizer can range from a

value of -9 to a value of +9, and the sum-to-memory value at each time point can range from -99 to +99.

time (μs)	80	160	240	320	400	480	...
FID1	8	3	0	-2	-3	-1	...
memory	**8**	**3**	**0**	**-2**	**-3**	**-1**	**...**
FID2	7	2	0	-1	-3	0	...
memory	**15**	**5**	**0**	**-3**	**-6**	**-1**	**...**
FID3	9	3	1	-3	-2	-1	...
memory	**24**	**8**	**1**	**-6**	**-8**	**-2**	**...**
FID4	8	3	-1	-1	-4	0	...
memory	**32**	**11**	**0**	**-7**	**-12**	**-2**	**...**

Thus in this case the receiver would overflow ("clip") with any FID value greater than +9 or less than -9. Notice that the FID values for different scans at any given time point are roughly the same, since only the noise is different. At each time point the FID value is added to the running total in memory; for example, the 160 μs time point of FID3 has a value of 3, which is added to the previous sum value of 5 to give the new sum value of 8. As more and more FIDs are acquired the sum increases steadily and will overflow the number of digits allotted to it in memory after a certain number of scans (sum greater than 99 or less than -99). On Varian instruments this will stop acquisition, resulting in the error message "maximum number of transients accumulated". This will only occur on long (e.g., overnight) acquisitions and can be avoided by setting the variable **DP** (double precision) to **Y**. This doubles the number of digits used in memory (from 16 to 32 binary digits), and also doubles the size of the data file. On the Bruker a memory overflow (beyond the 24 binary digits reserved) results in the whole FID sum in memory being divided by two; acquisition continues with the new FIDs being divided by two before being added in. In this way Bruker never has a problem with memory overflow, but accuracy is lost in the division process.

The number of scans is primarily determined by the concentration of the sample and the desired signal-to-noise ratio. Another factor to consider is the **phase cycle**. Artifacts which are inherent in the electronics of the spectrometer can be cancelled out by changing the phase of the RF pulse in a fixed pattern (e.g., 0^o, 90^o, 180^o, and 270^o in scans 1, 2, 3, and 4) and changing the phase of the receiver (by subtracting the signal instead of adding, or switching the real and imaginary parts) to follow this progression. The number of scans should be an integer multiple of the phase cycle length (a multiple of four for simple 1D acquisition) to assure optimal cancellation of artifacts. Some experiments, which subtract undesired signals from desired ones, will not work if the number of scans is set wrong. The phase cycle cancellation can also be screwed up if the first scan or two are acquired with the nuclei not in the “steady state” in terms of relaxation. Often the relaxation delay is not long enough for complete return of all spins to the equilibrium state, so the spins reach a steady state after a few scans where the degree of relaxation is always the same at the start of each scan. This steady-state can be established by using dummy scans (steady-state) scans. These are scans which include a Relaxation Delay, Pulse, and Acquisition just like a normal scan, but the data is not added into memory. The number of dummy (steady-state) scans is **DS** (Bruker) or **SS** (Varian).

Dictionary of NMR Parameters. What follows is a list of the most important NMR acqusition parameters, with their names in both "Brukerese" and "Varianese" and a brief explanation. Keep in mind that changing an acquisition parameter has no effect on the data (FID or spectrum) unless you repeat the acquisition with the new parameter setting.

Spectral Width: **SW** (Bruker and Varian). The spectral width is the width of the spectral window in Hz, from the left edge of the spectrum to the right edge of the spectrum. Thus, to "cover" a proton chemical shift range of 10 ppm to -1 ppm on a 300 MHz spectrometer, we need a spectral width of 11 ppm, which is 11 X 300 or 3,300 Hz. Any peak which is more than 5.5 ppm (1,650 Hz) from the center of the spectral window (4.5 ppm) will be incorrectly determined as fas as frequency is concerned. The tricky thing is that the peak is not simply ignored; it appears in your spectrum with an erroneous frequency, and usually an erroneous phase as well. These "aliased" peaks can be eliminated simply by increasing the spectral width and trying again. Continuing with this example, a spectral width of 3,300 Hz requires that we collect a real, imaginary pair of data points every 30.3 μs (Varian), or a single data point every 15.15 μs (Bruker). To collect 16,384 data points (8,192 pairs) will take an acquisition time of:

$$\mathbf{AT} = \mathbf{NP} / (2 * \mathbf{SW}) = 16{,}384 / (2 * 3{,}300) = \mathbf{2.482} \text{ seconds}$$

Acquisition Time: **AT** (Varian) or **AQ** (Bruker). The length of time required to collect the data of a single FID. Be careful not to confuse this with the recycle time, which also include the pulse width (**PW**) and the relaxation delay (**D1** or **RD**), or with the length of time for the entire experiment, is the number of repeats times the recycle time. If you change the spectral width (**SW**), Bruker will automatically change the acquisition time (**AQ**), but Varian will keep acquisition time (**AT**) constant and change the number of points (**NP**). The acquisition time should be long enough to include the full decay of the FID into noise; any longer time will only decrease the signal-to-noise ratio of your spectrum since you are collecting only noise.

Number of Data Points: **NP** (Varian) or **TD** (Bruker). The total number of data points (real or imaginary) in an FID. There is no confusion here between Varian and Bruker on the definition, but some software packages for NMR data processing (e.g., Felix), measure the size of the data set in terms of the number of pairs (real, imaginary) of data points. Both Bruker and Varian provide for the possibility of enlarging the data set by adding zeros after the actual data ("zero-filling") before the Fourier transform. This is done to give better definition of peak shapes by using more points to define the spectrum. Thus, the total number of data points before the Fourier transform (including these zeros) is called **SI** ("size", Bruker) or **FN** ("Fourier number", Varian). If you save your data to the disk, the size of the file will be directly proportional to the number of points.

Offset: **TO** (Varian) or **O1** (Bruker). The offset (or transmitter offset) sets the position of the center of the spectral window. For example, if the normal spectral window for ^{1}H on a 200 MHz instrument extends from 11 ppm to -1 ppm and you wanted to instead cover the region 13 ppm to 1 ppm, you would simply add 400 Hz (2 ppm) to the value of **TO** (or **O1** on the Bruker). The offset represents a correction (in Hz) added to the generic radio frequency (e.g.,

200.000 MHz) of the particular nucleus being observed. All spectrometers have a second source of RF power called the decoupler, and the offset of the decoupler is **DO** (Varian) or **O2** (Bruker). This represents the center of the range of frequencies you are trying to decouple; for example, in a ^{1}H-decoupled ^{13}C spectrum **DO** (**O2**) would be the center of the proton spectrum and **TO** (**O1**) would be the center of the (^{13}C) spectral window. The position of the center of your spectrum in ppm will depend not only on the offset, but also on the ^{2}H chemical shift of your lock solvent, since this is necessarily the only reference the spectrometer has. To correct for this, Varian has a parameter ("**SOLVNT**") which corrects the offset for the different ^{2}H chemical shifts of various lock solvents. Using the Bruker, you can simply call up different parameter files (e.g., CARBON.CHL, CARBON.D2O, CARBON.ACT) which will each have the correct **O1** and **O2** values for that particular lock solvent.

Number of Transients or Scans: **NT** (Varian) or **NS** (Bruker). This is the number of repeats of the **D1** - **PW** - **AT** (**RD** - **PW** - **AQ**) sequence (Fig. 14). Obviously, the total time required by the experiment is proportional to this number. The number of scans completed at any given time is displayed on the screen as the acquisition proceeds. You can stop the acquisition prematurely after any number of scans (**/A** on the Varian, or **<control> H** in Bruker) and process the summed FID available up to that point. The Varian actually saves the new FID sum to disk every time a "block" of transients is completed. The number of transients in a block is set by the parameter **BS** (Block Size). For example, if **BS** is set to 16 and you abort aquisition after completion of the 38th transient, the summed FID you get will include only 32 scans (two completed blocks). On the Bruker you can set **NS** to -1, meaning that it will continue acquiring forever until you tell it to stop. The same thing can be accomplished on Varian by setting **NT** to a very large number. To determine the length of time required to finish the requested number of scans, enter **TIME** (Varian) or **EXPT** (Bruker).

Receiver Gain: **GAIN** (Varian) or **RG** (Bruker). This is the amount of amplification the FID signal is given both in the RF stage and in the audio frequency stage. The gain must be adjusted for the concentration of each sample in ^{1}H NMR so that the final signal coming into the digitizer is almost filling but not overflowing the fixed digitizer limits. A gain setting too low will reduce signal-to-noise ratio (sensitivity) and dynamic range (the ability to detect small signals in the presence of large ones). A gain setting too large will lead to "clipping" (cutting off) of the most intense part of the FID, which will give a wavy baseline in the transformed spectrum. The gain can be set automatically by setting **GAIN = N** on the Varian, or by using the command **RGA** (receiver gain adjust) on the Bruker. On the Varian Gemini-200 it is possible to overflow the digitizer with very concentrated samples even with the **GAIN** set at the lowest possible value. In this case the pulse width (**PW**) should be drastically reduced (e.g., **PW = 3**) to further reduce the FID signal. For ^{13}C-NMR the receiver gain is usually set to the maximum value, and it is very rare that a sample can overflow the digitizer even at this setting.